FIG. 1

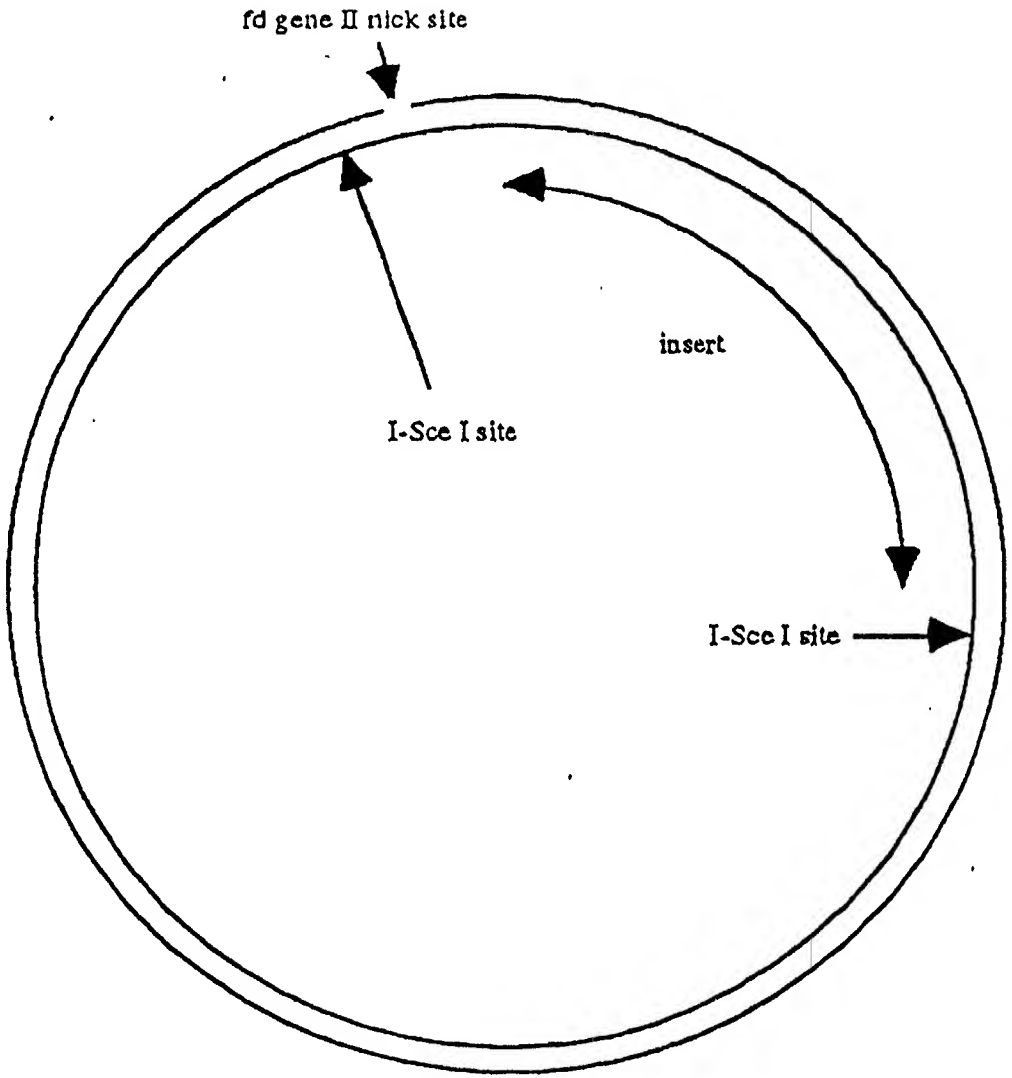


FIG. 2

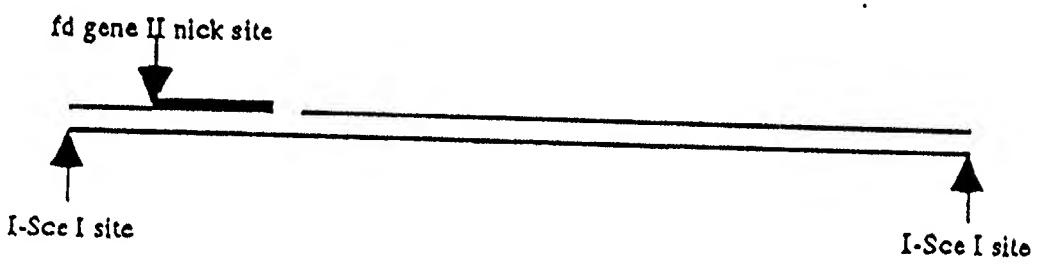
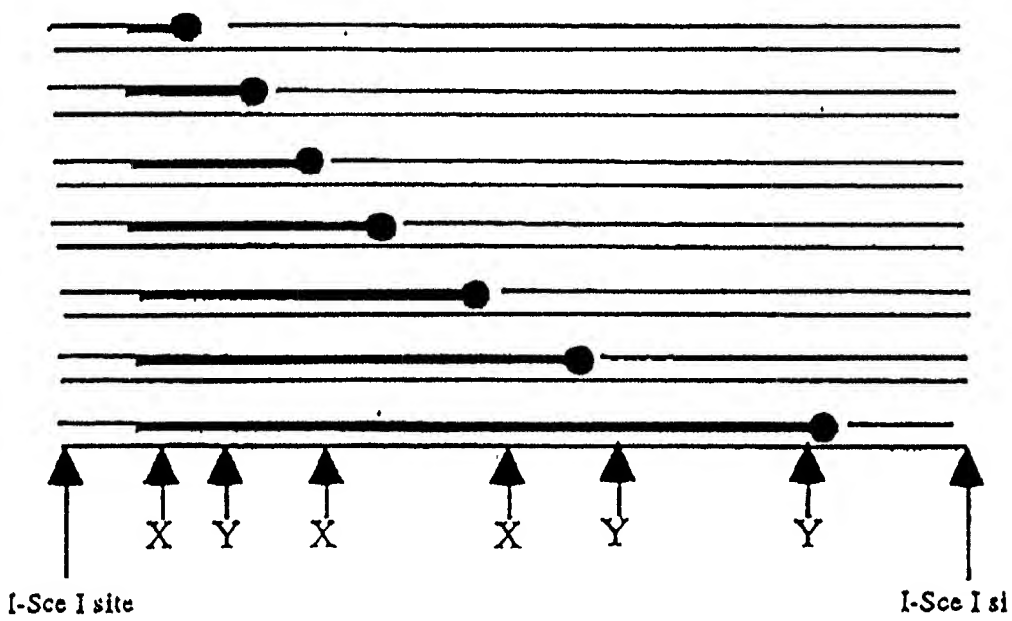


FIG. 3



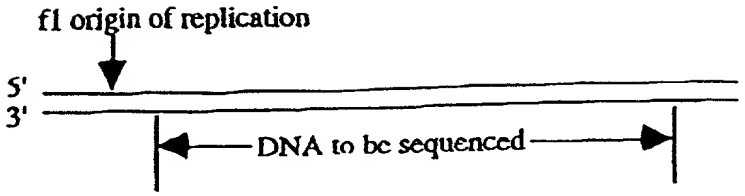


FIG. 4A

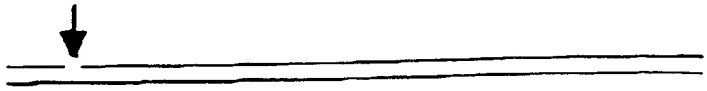


FIG. 4B

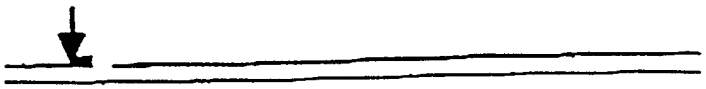


FIG. 4C

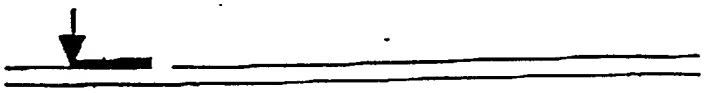


FIG. 4D

FIG. 4E

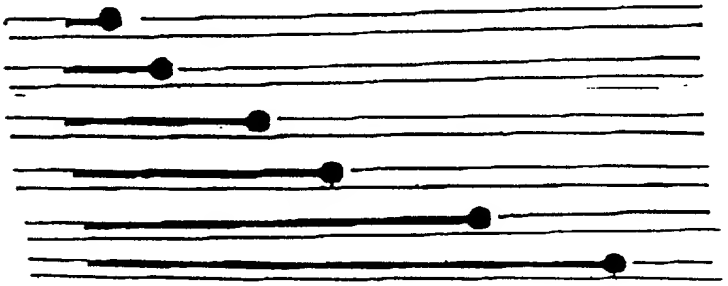


FIG. 4F

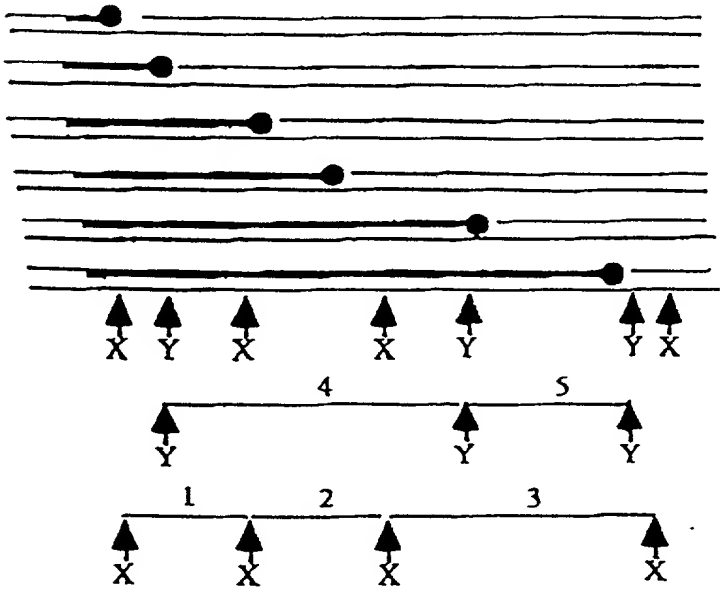


FIG. 4G

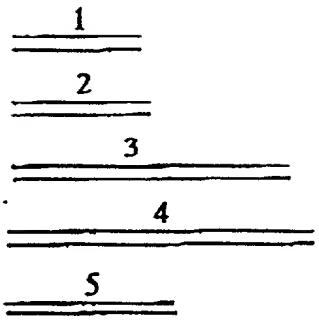


FIG. 4H

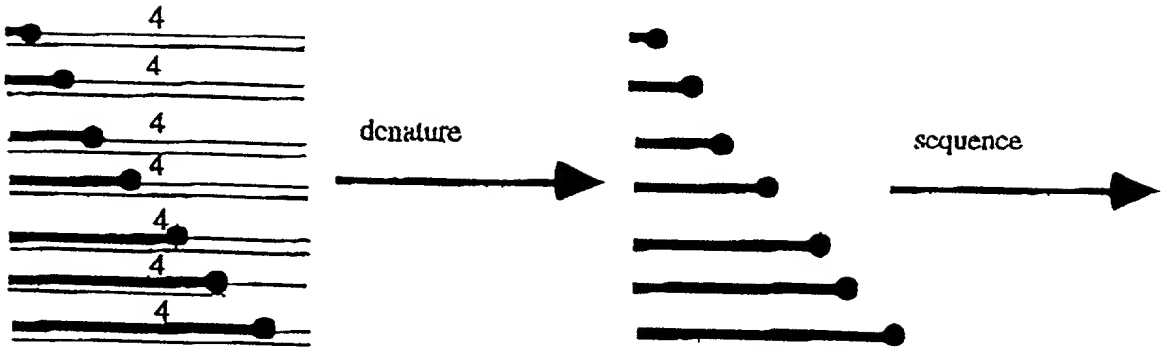


FIG. 4I

FIG. 5

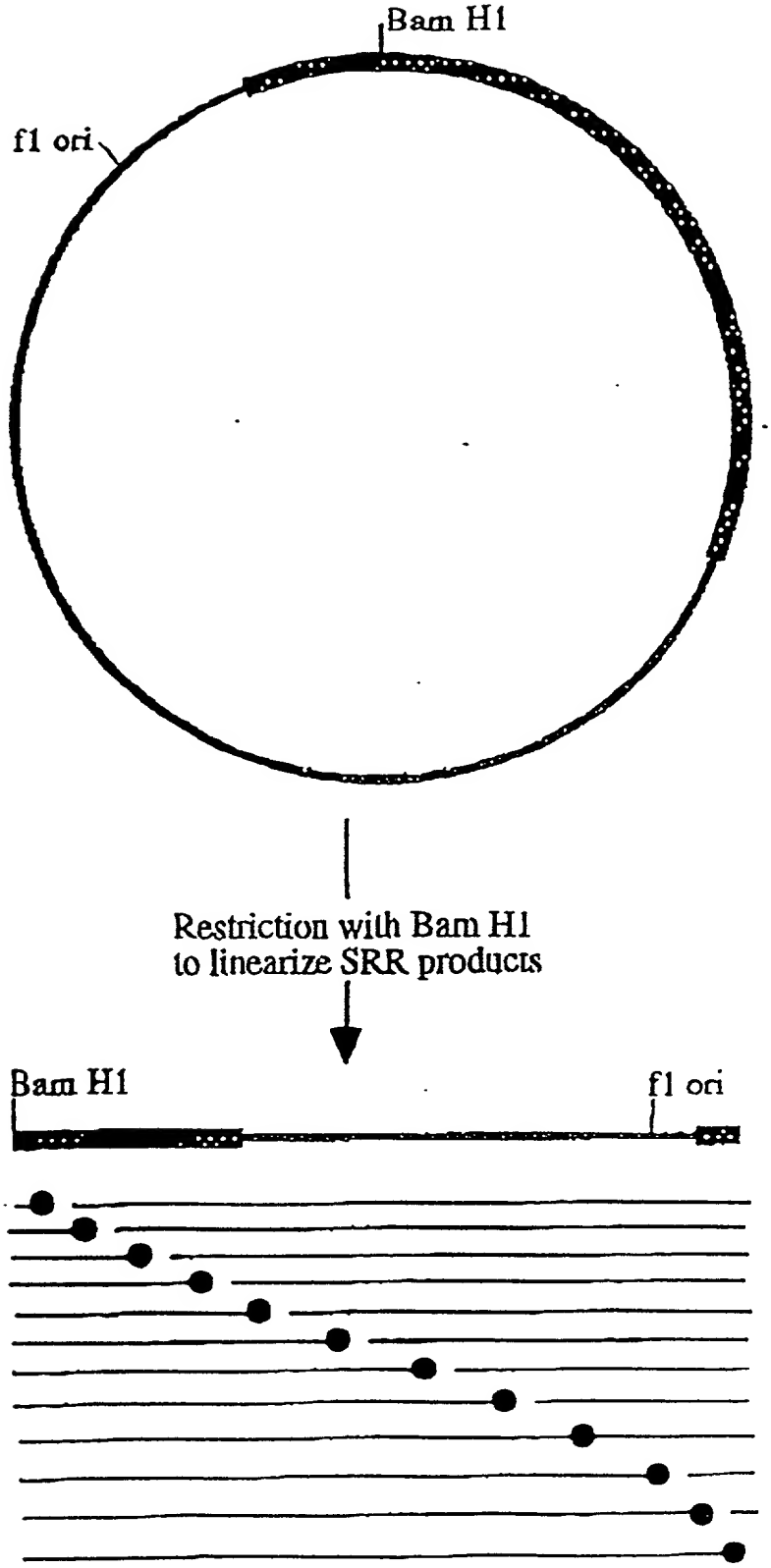

Bam H1
f1 ori
Restriction with Bam H1
to linearize SRR products
Bam H1
f1 ori

FIG. 6

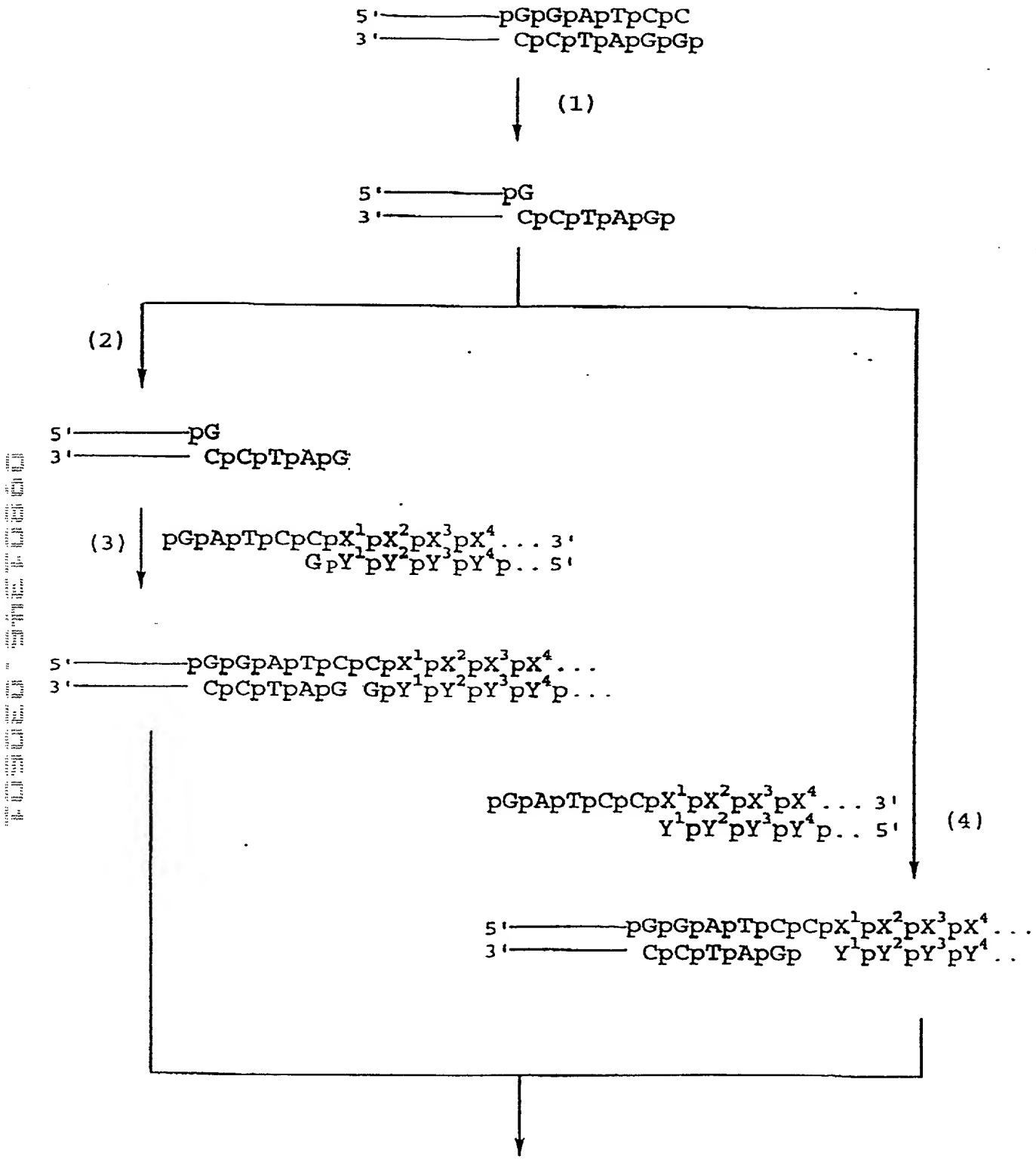

5'——pGpGpApTpCpC
3'—— CpCpTpApGpGp
(1)
5'——pG
3'—— CpCpTpApGp
(2)
5'——pG
3'—— CpCpTpApG
(3)
pGpApTpCpCpX1pX2pX3pX4... 3'
GpY1pY2pY3pY4p.. 5'
5'——pGpGpApTpCpCpX1pX2pX3pX4...
3'—— CpCpTpApG GpY1pY2pY3pY4p...
pGpApTpCpCpX1pX2pX3pX4... 3'
Y1pY2pY3pY4p.. 5'
(4)
5'——pGpGpApTpCpCpX1pX2pX3pX4...
3'—— CpCpTpApGp Y1pY2pY3pY4..
STRAND REPLACEMENT REACTION

FIG. 7A

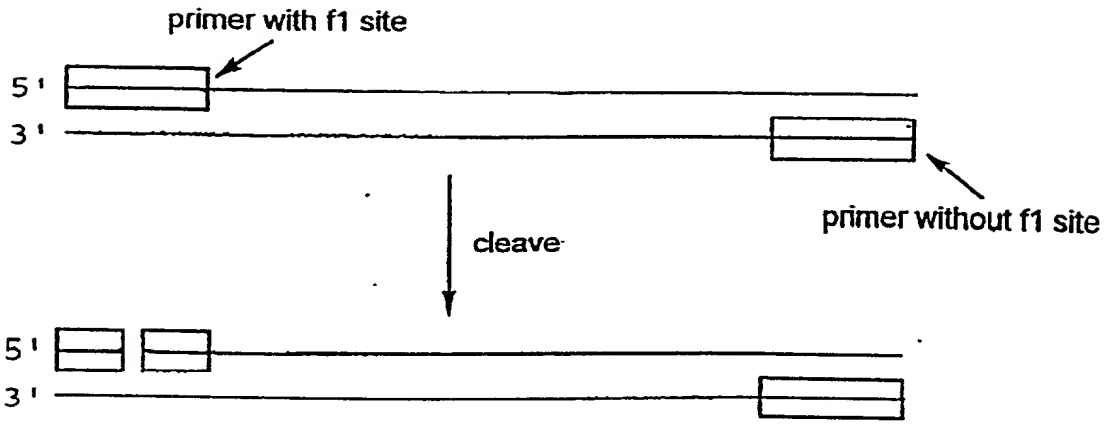

primer with f1 site
5'
3'
primer without f1 site
cleave
5'
3'

FIG. 7B

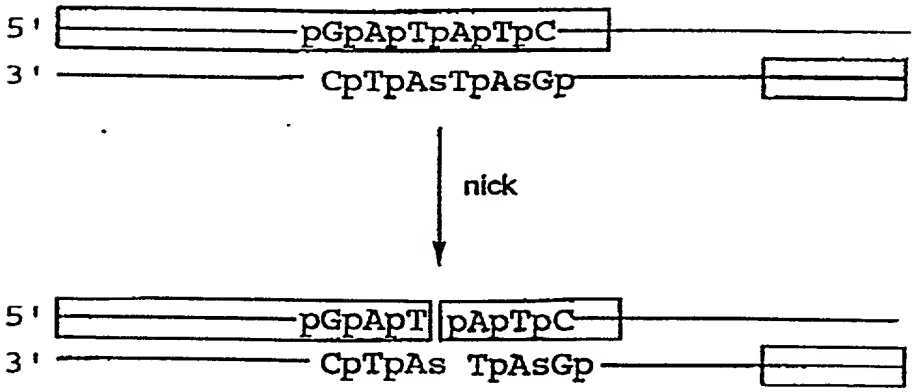

5'
pGpApTpApTpC
3'
CpTpAsTpAsGp
nick
5'
pGpApT
pApTpC
3'
CpTpAs TpAsGp

FIG. 7C

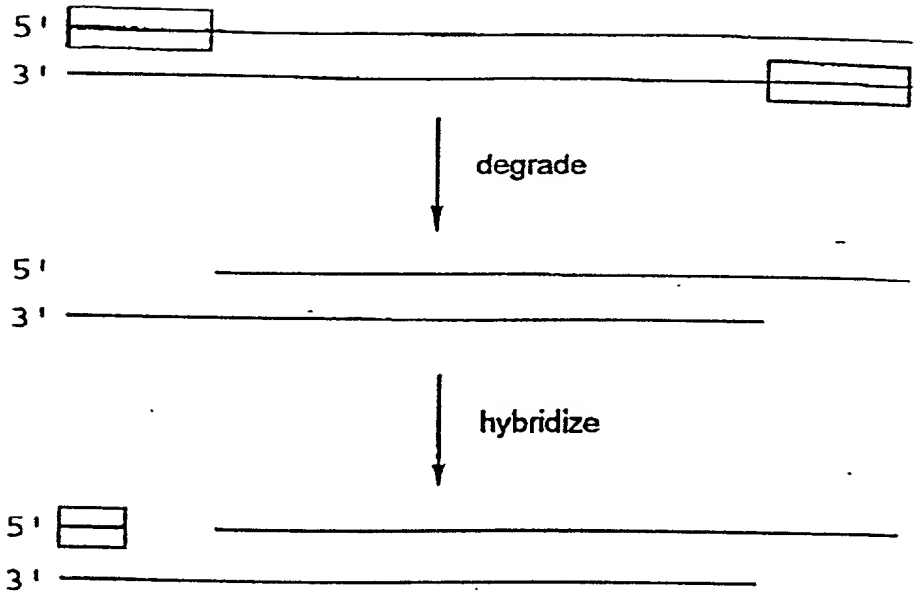
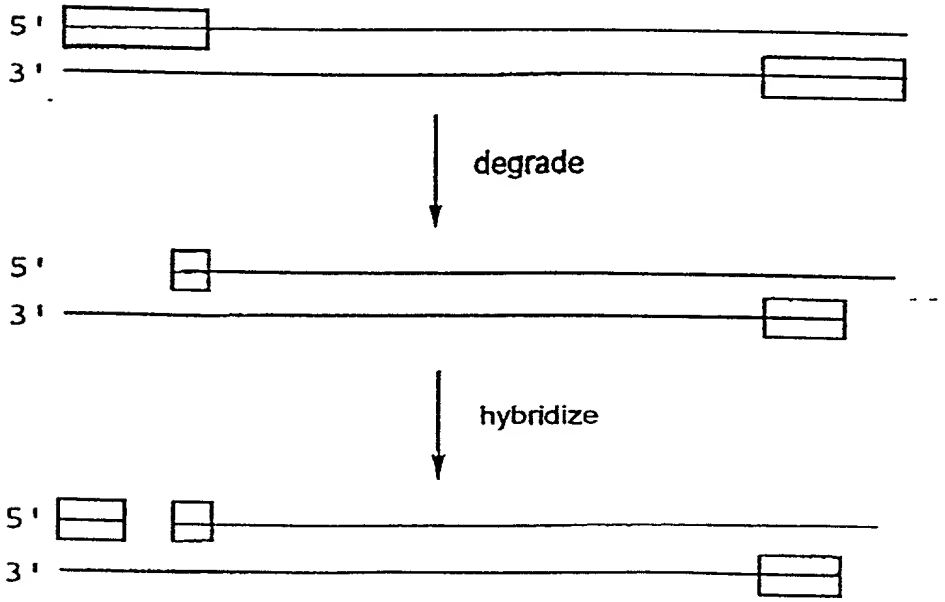


FIG. 7D



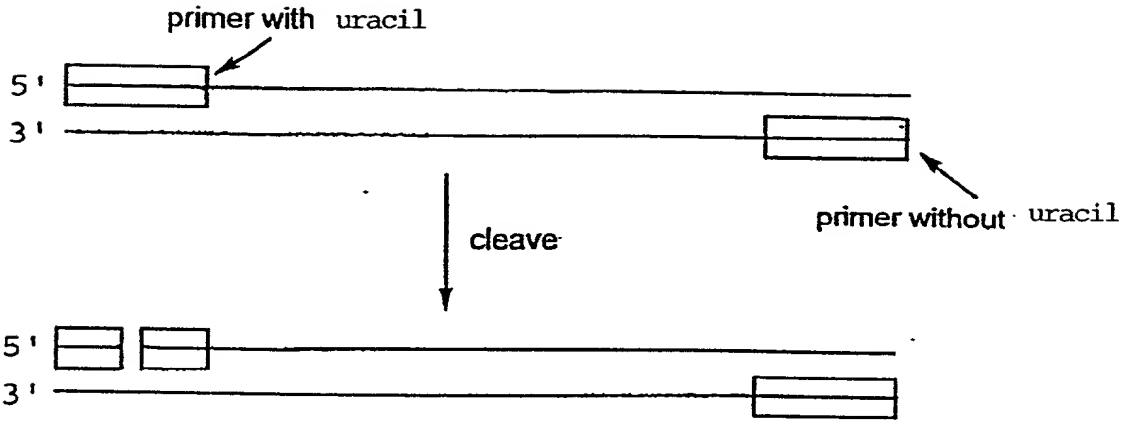


FIG. 7E

FIG. 8

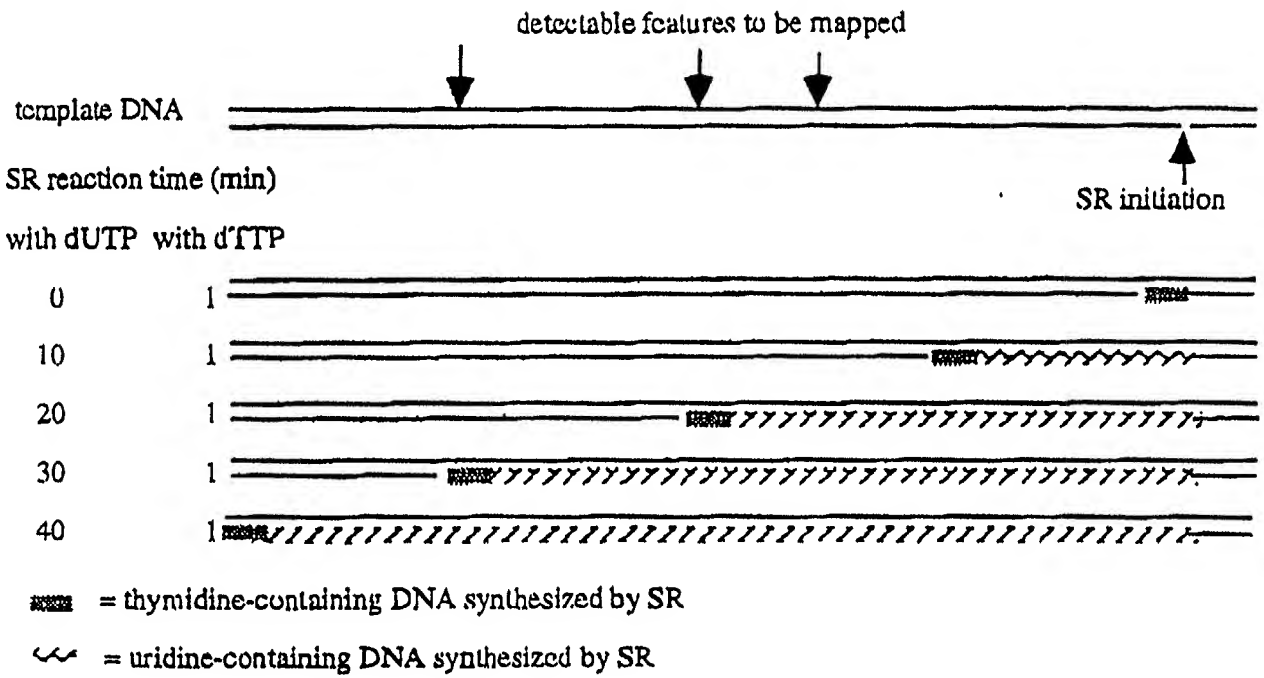


FIG. 9

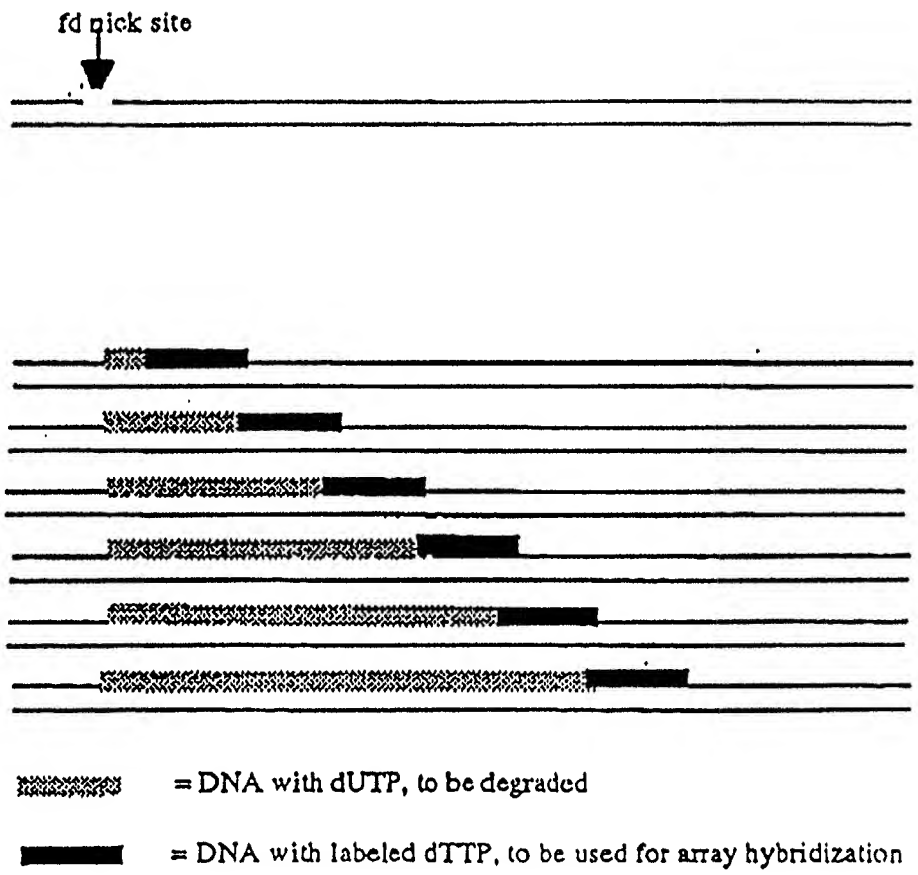


FIG. 10A

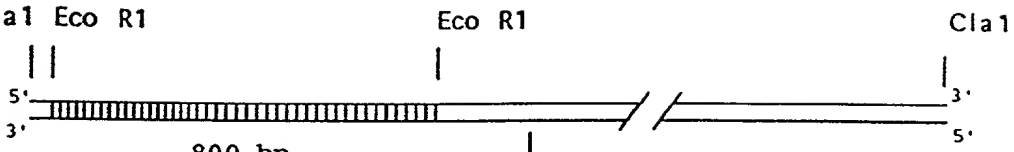


FIG. 10B

FIG. 10C

FIG. 10D

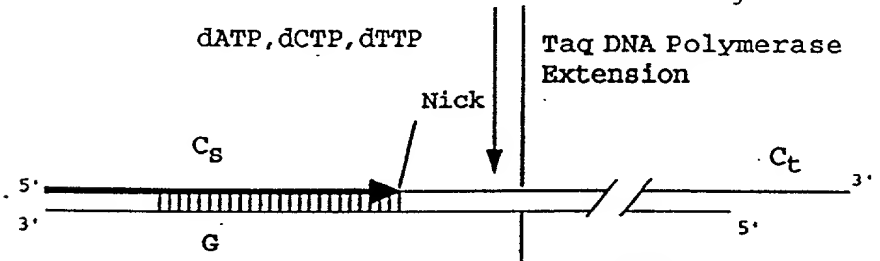


FIG. 10E

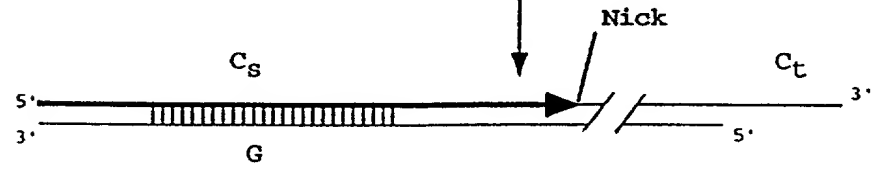


FIG. 11

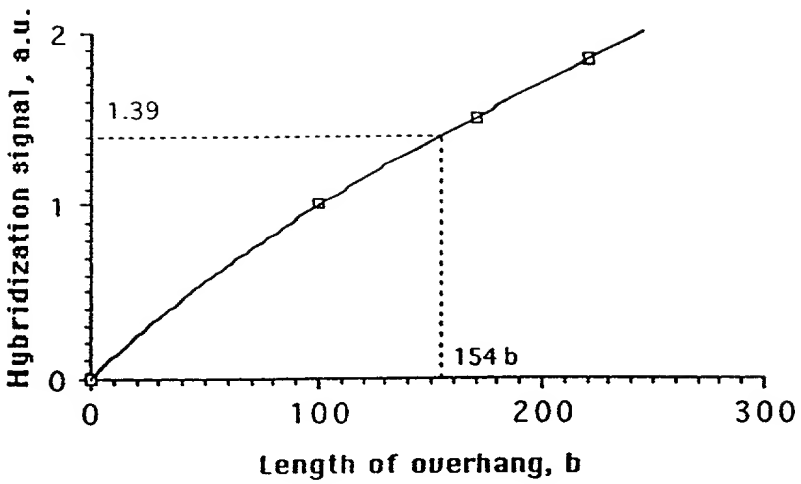
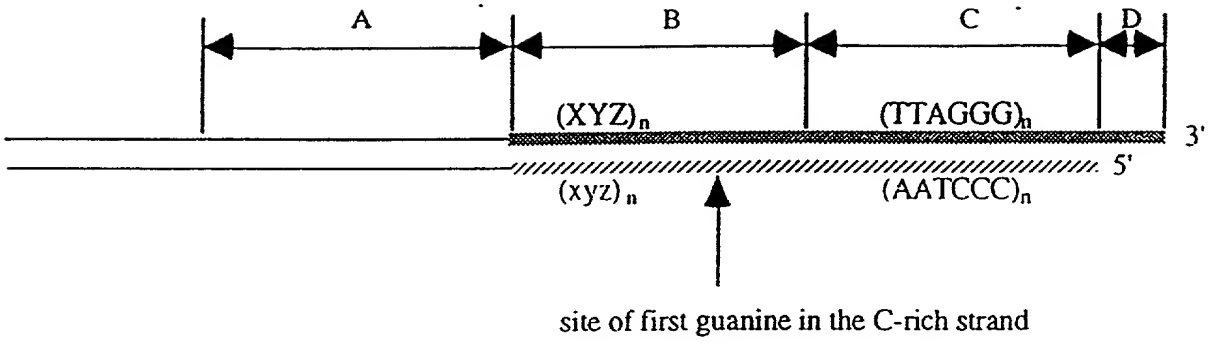

Hybridization signal, a.u.
2
1
0
1.39
154 b
0
100
200
300
Length of overhang, b

FIG. 12



reaction time (min)

10

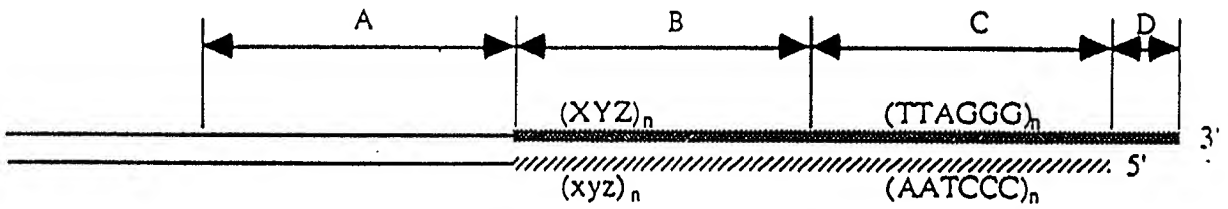
20

30

40

//// = DNA synthesized by PENT using only dATP, dTTP, and dCTP

FIG. 13



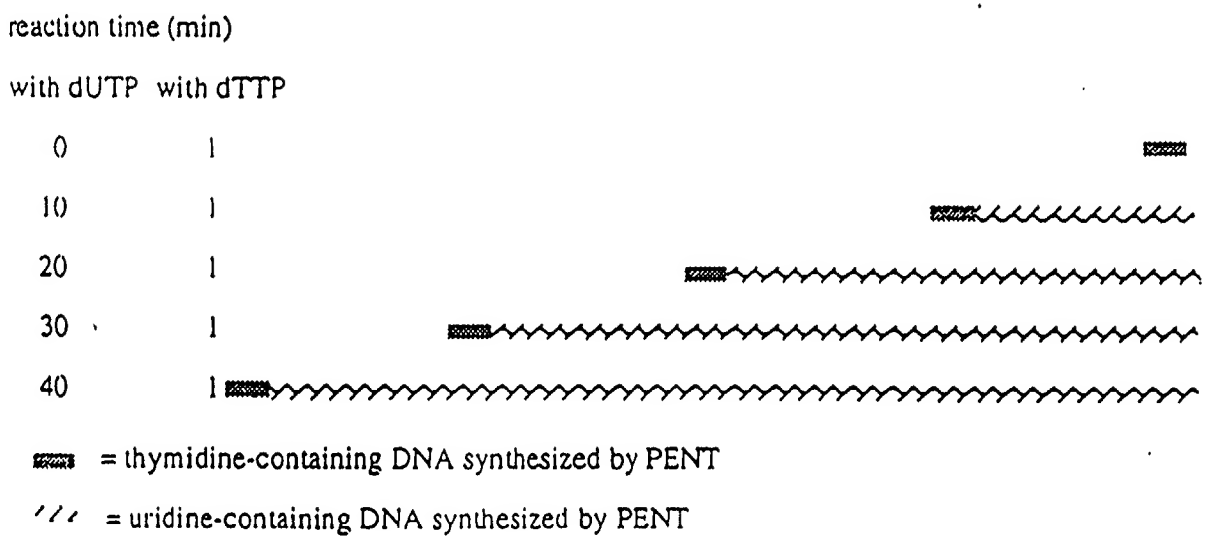


FIG. 14A

FIG. 14B

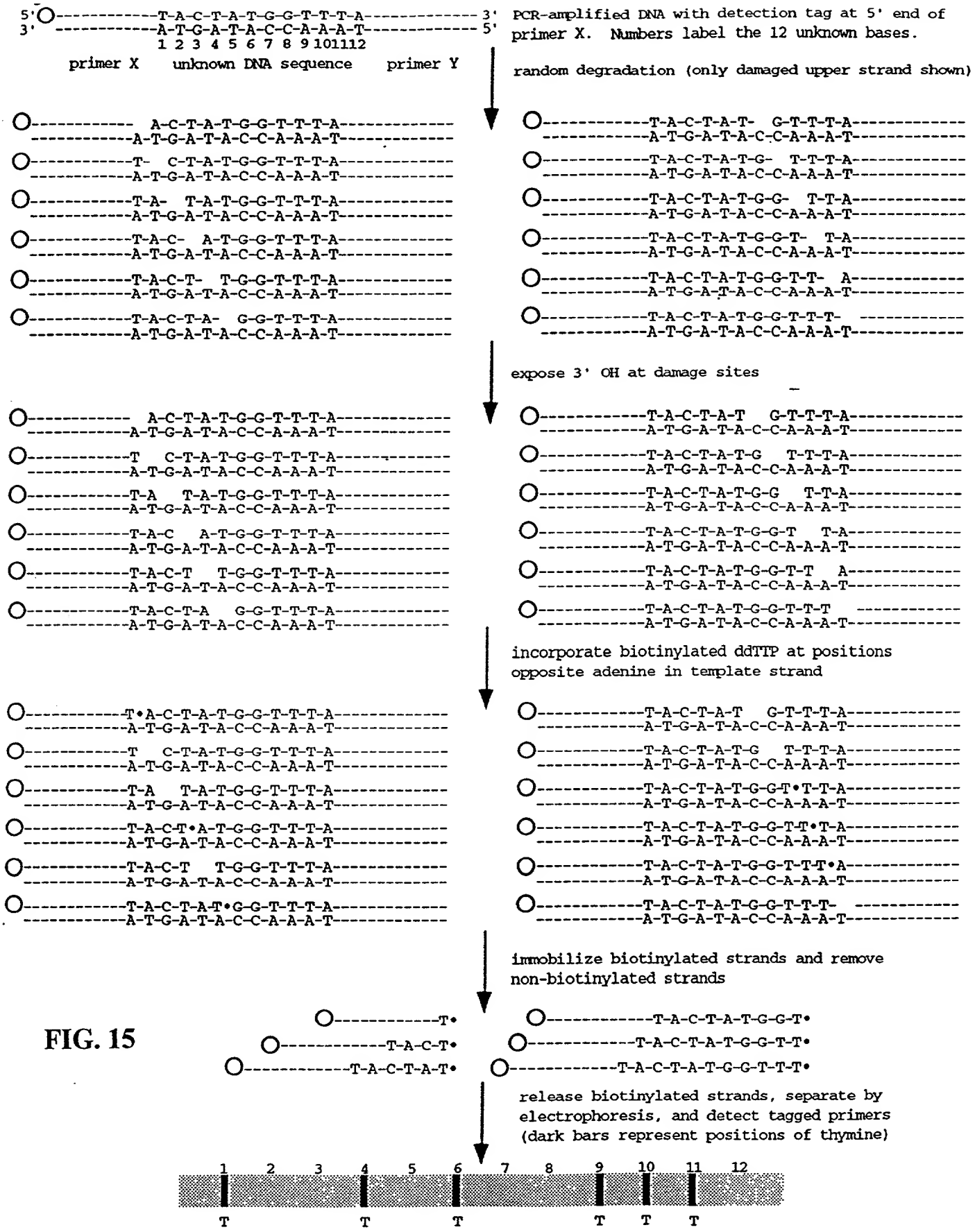
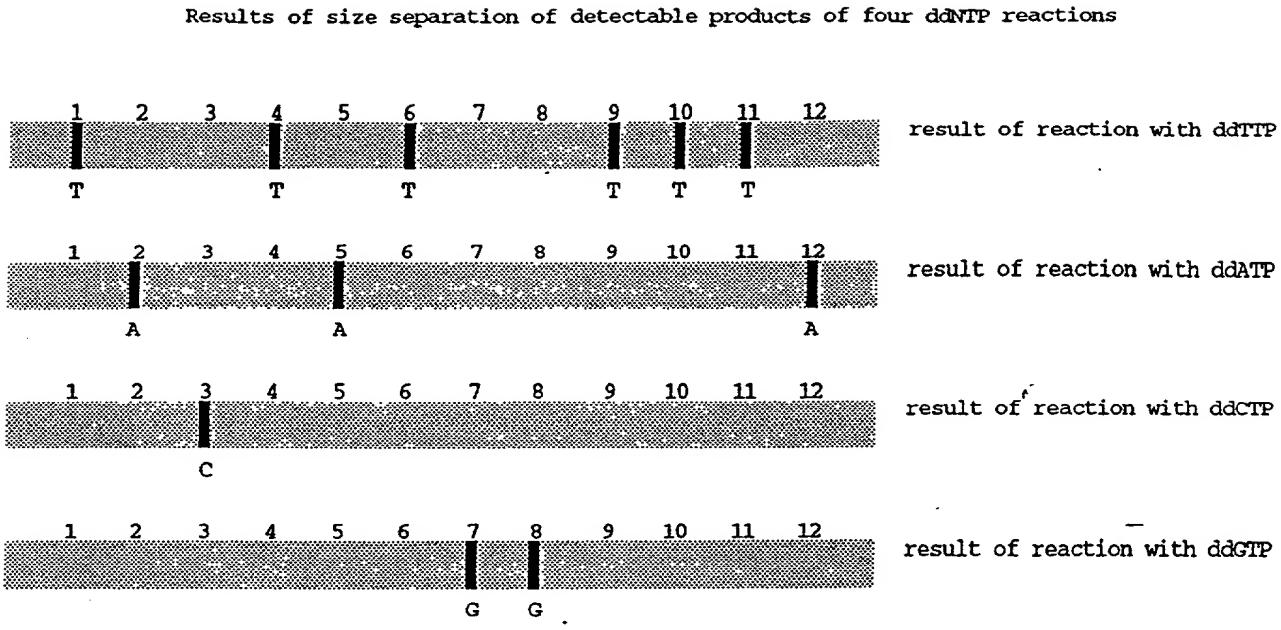

5'-T-A-C-T-A-T-G-G-T-T-T-A- 3'
3'-A-T-G-A-T-A-C-C-A-A-A-T- 5'
1 2 3 4 5 6 7 8 9 10 11 12
primer X
unknown DNA sequence
primer Y
PCR-amplified DNA with detection tag at 5' end of primer X. Numbers label the 12 unknown bases.
random degradation (only damaged upper strand shown)
expose 3' OH at damage sites
incorporate biotinylated ddTTP at positions opposite adenine in template strand
immobilize biotinylated strands and remove non-biotinylated strands
release biotinylated strands, separate by electrophoresis, and detect tagged primers (dark bars represent positions of thymine)
T
T
T
T
T
T

FIG. 15


Results of size separation of detectable products of four ddNTP reactions
1 2 3 4 5 6 7 8 9 10 11 12
T T T T T T
result of reaction with ddTTP
1 2 3 4 5 6 7 8 9 10 11 12
A A A
result of reaction with ddATP
1 2 3 4 5 6 7 8 9 10 11 12
C
result of reaction with ddCTP
1 2 3 4 5 6 7 8 9 10 11 12
G G
result of reaction with ddGTP

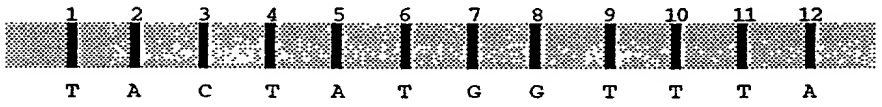

summation of ddNTP results into complete base sequence
1 2 3 4 5 6 7 8 9 10 11 12
T A C T A T G G T T T A

FIG. 16

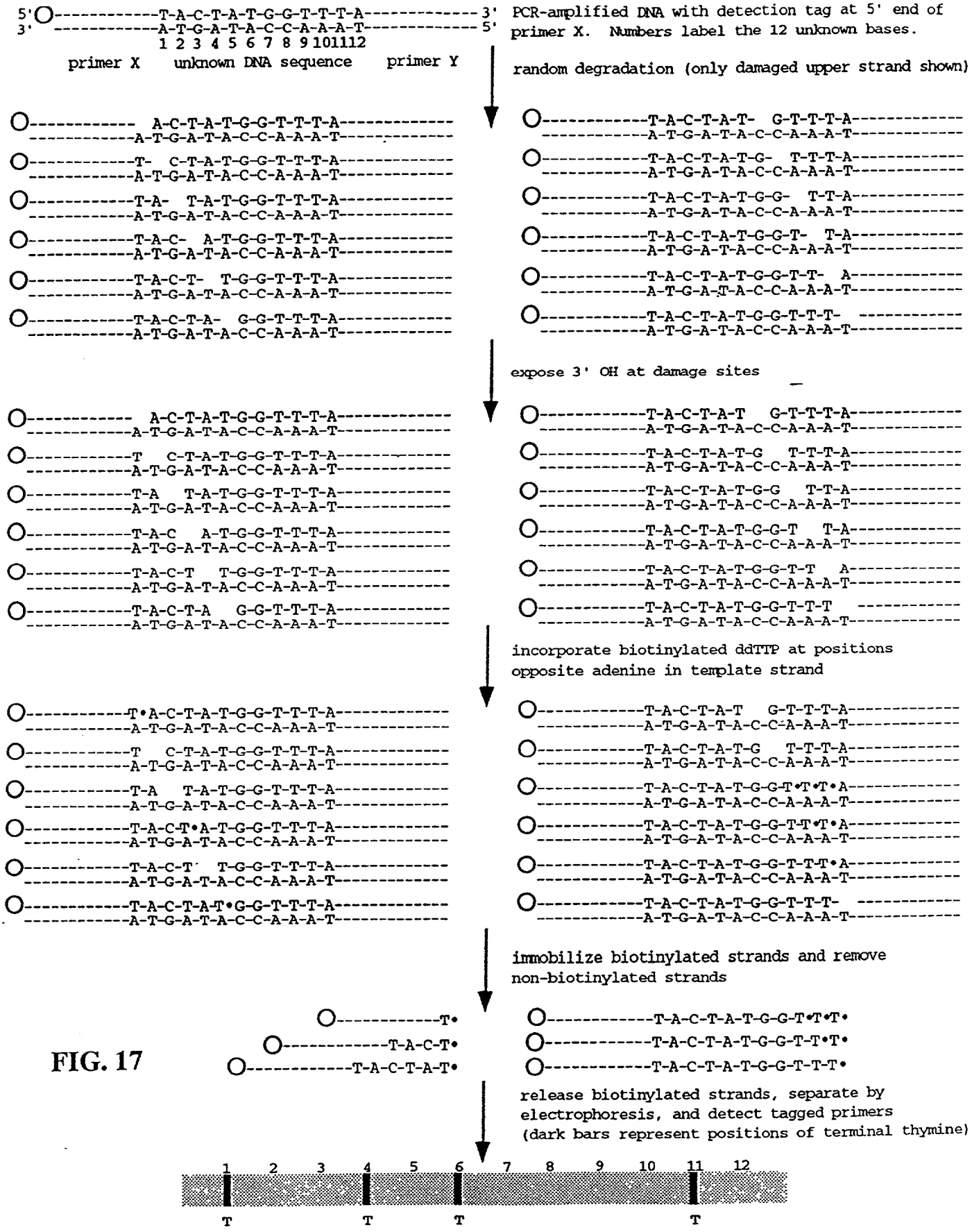
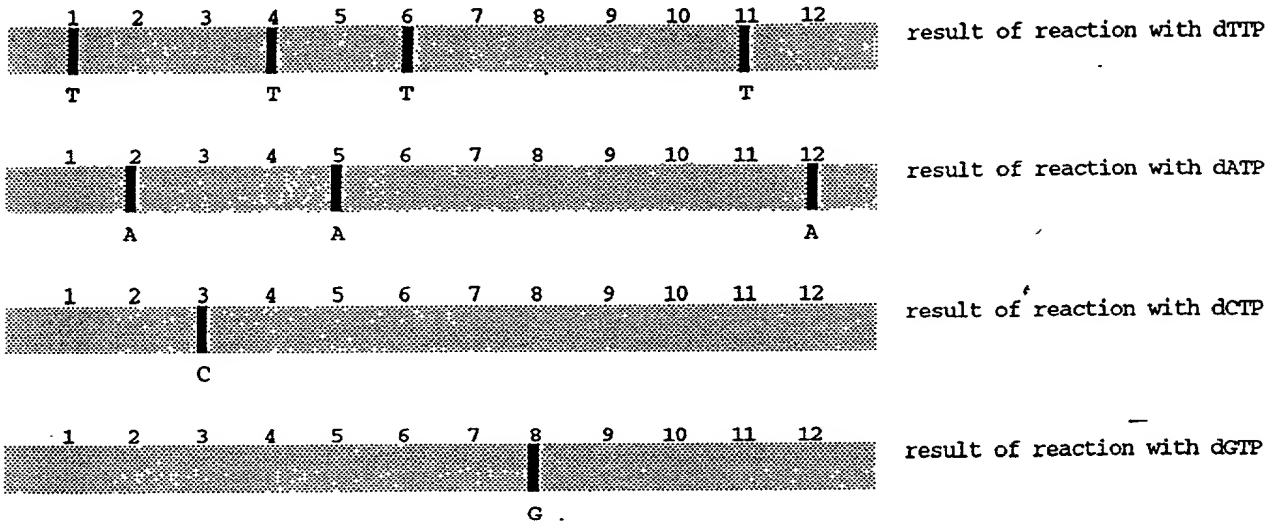

5' O----------T-A-C-T-A-T-G-G-T-T-T-A---------- 3'
3' ----------A-T-G-A-T-A-C-C-A-A-A-T---------- 5'
1 2 3 4 5 6 7 8 9 10 11 12
primer X
unknown DNA sequence
primer Y
PCR-amplified DNA with detection tag at 5' end of primer X. Numbers label the 12 unknown bases.
random degradation (only damaged upper strand shown)
expose 3' OH at damage sites
incorporate biotinylated ddTTP at positions opposite adenine in template strand
immobilize biotinylated strands and remove non-biotinylated strands
release biotinylated strands, separate by electrophoresis, and detect tagged primers (dark bars represent positions of terminal thymine)
T
T
T
T

FIG. 17

Results of size separation of detectable products of four dNTP reactions



Summation of dNTP results into complete base sequence
(positions of bases in parentheses are inferred)

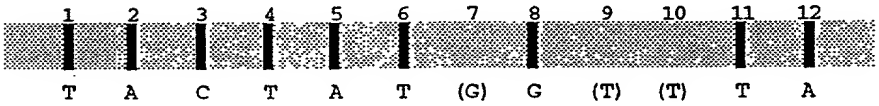


FIG. 18

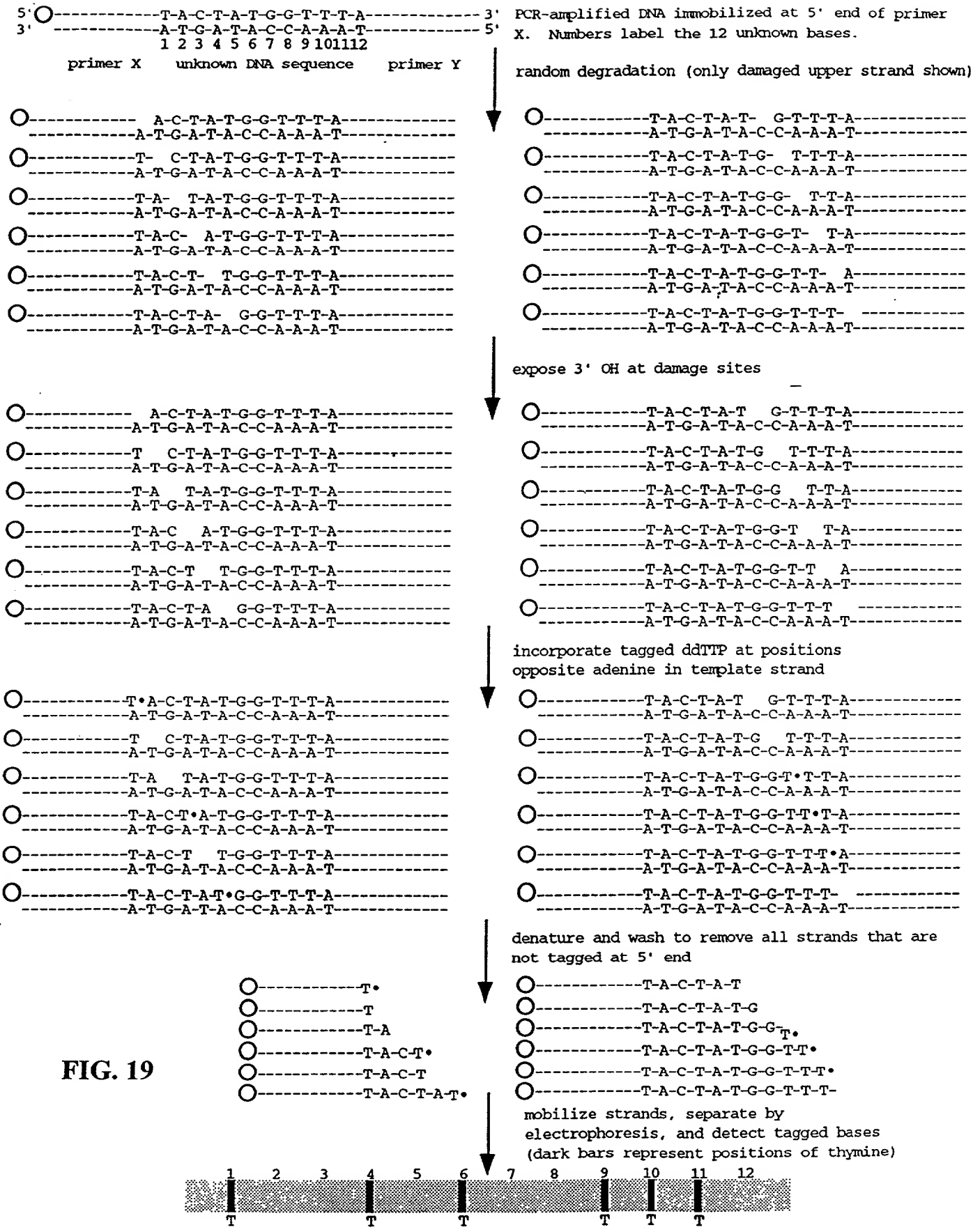

PCR-amplified DNA immobilized at 5' end of primer X. Numbers label the 12 unknown bases.
primer X
unknown DNA sequence
primer Y
random degradation (only damaged upper strand shown)
expose 3' OH at damage sites
incorporate tagged ddTTP at positions opposite adenine in template strand
denature and wash to remove all strands that are not tagged at 5' end
mobilize strands, separate by electrophoresis, and detect tagged bases (dark bars represent positions of thymine)
1 2 3 4 5 6 7 8 9 10 11 12
T T T T T T

FIG. 19

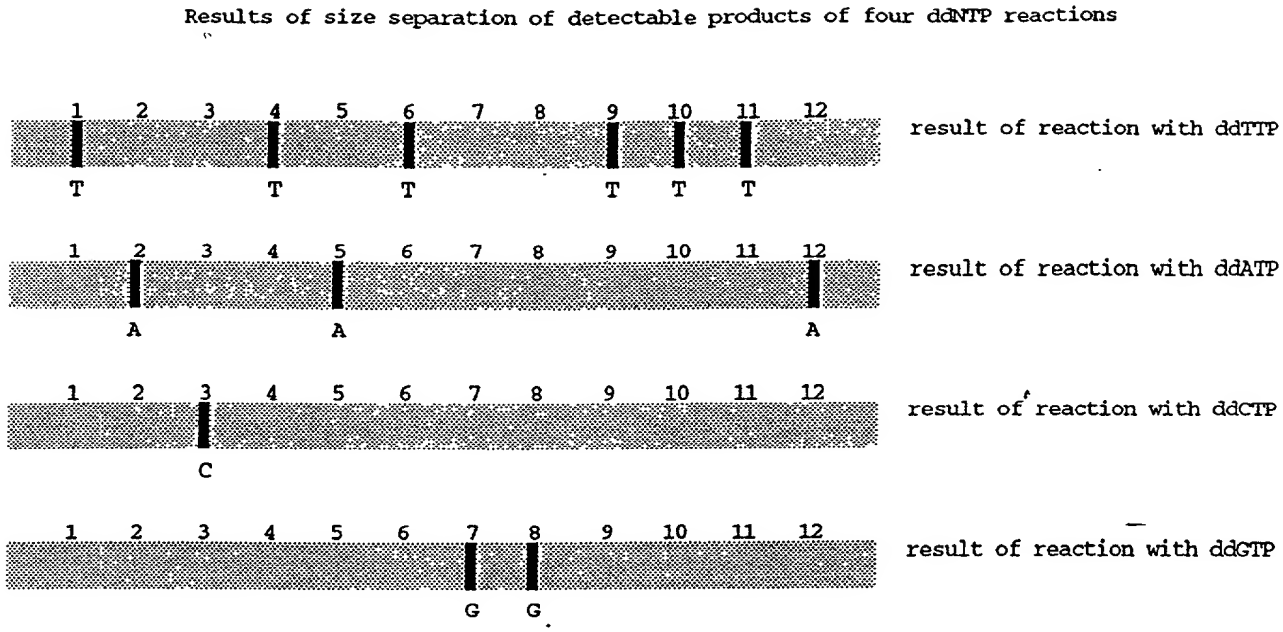

Results of size separation of detectable products of four ddNTP reactions
1 2 3 4 5 6 7 8 9 10 11 12
T T T T T T
result of reaction with ddTTP
1 2 3 4 5 6 7 8 9 10 11 12
A A A
result of reaction with ddATP
1 2 3 4 5 6 7 8 9 10 11 12
C
result of reaction with ddCTP
1 2 3 4 5 6 7 8 9 10 11 12
G G
result of reaction with ddGTP
summation of ddNTP results into complete base sequence
1 2 3 4 5 6 7 8 9 10 11 12
T A C T A T G G T T T A

FIG. 20

PCR amplify, immobilize, and expose 3' OH
at random sites as in Fig. 5.

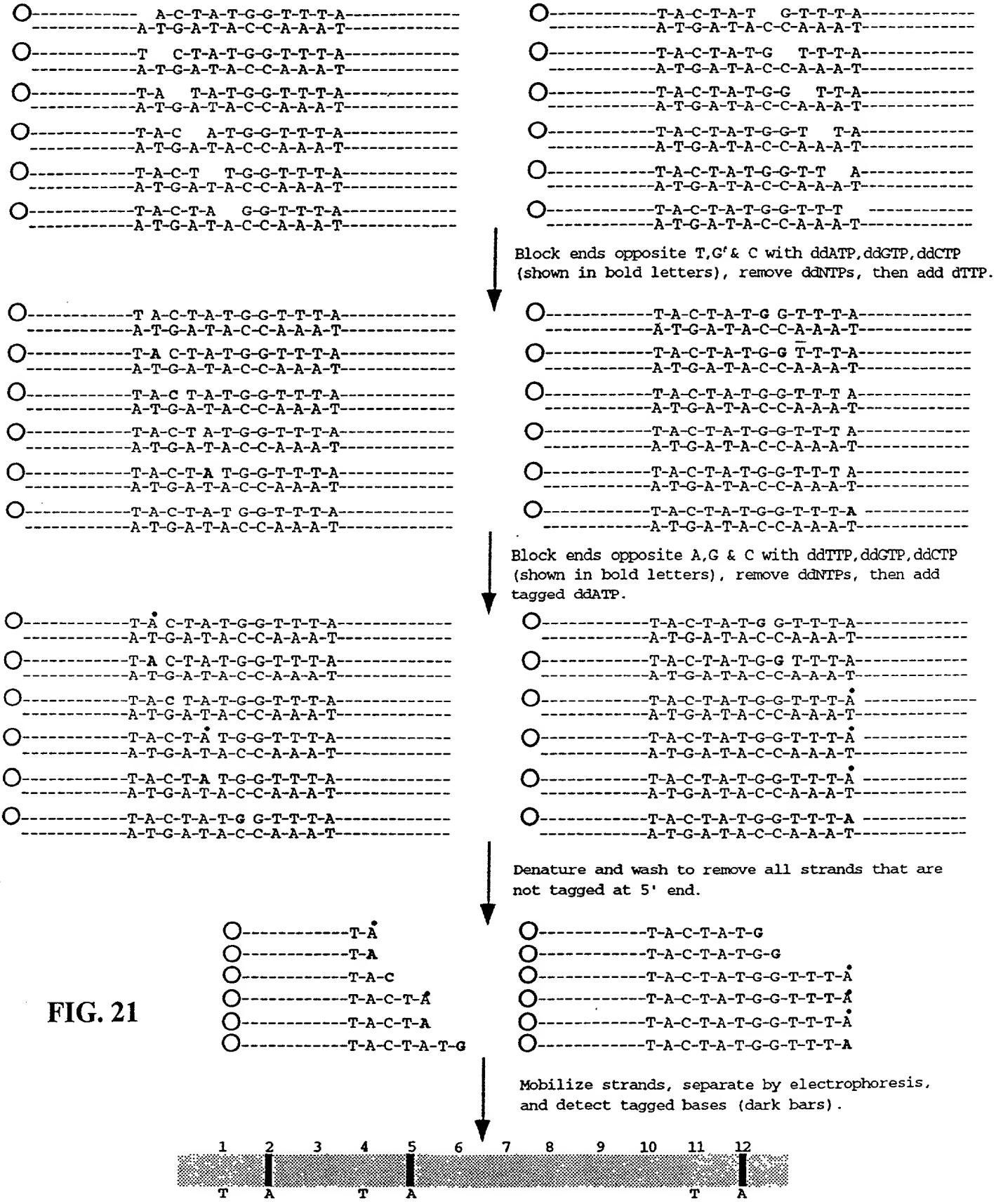
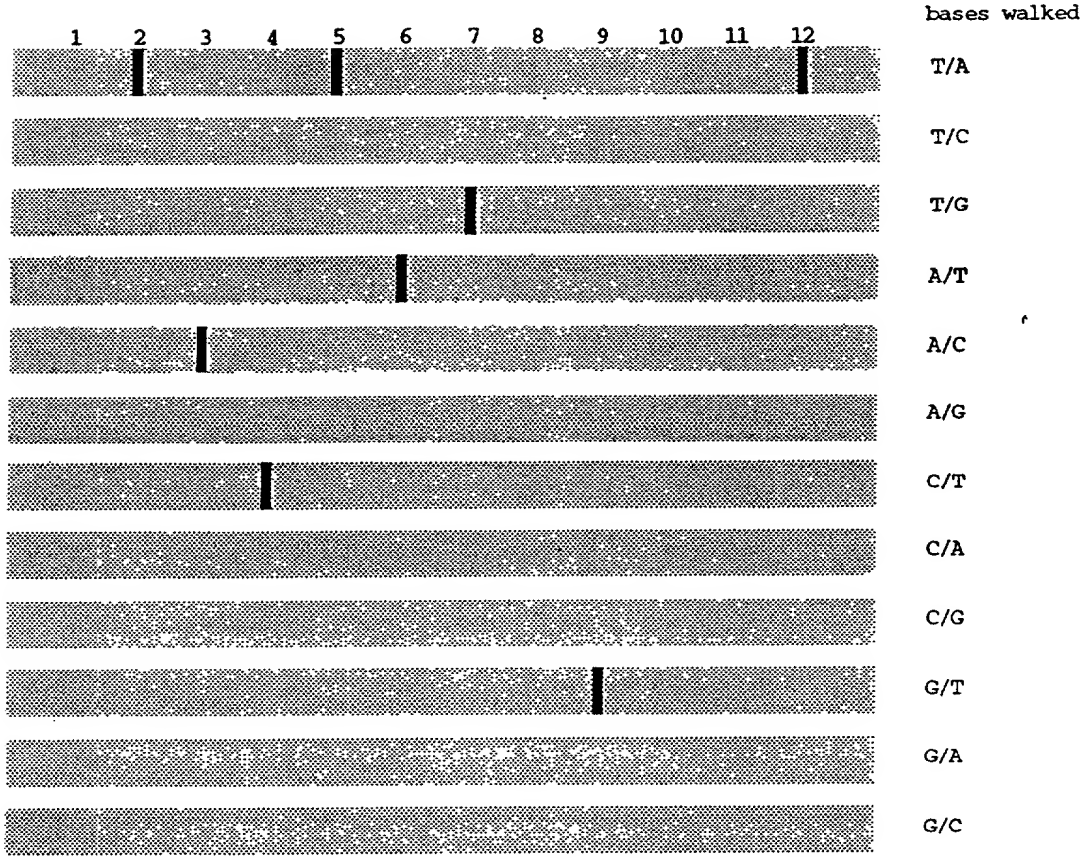


FIG. 21

Size separation of the products of twelve 2-base walk reactions



Assembly of complete sequence from the results of individual reactions
(inferred bases in parentheses)

FIG. 22

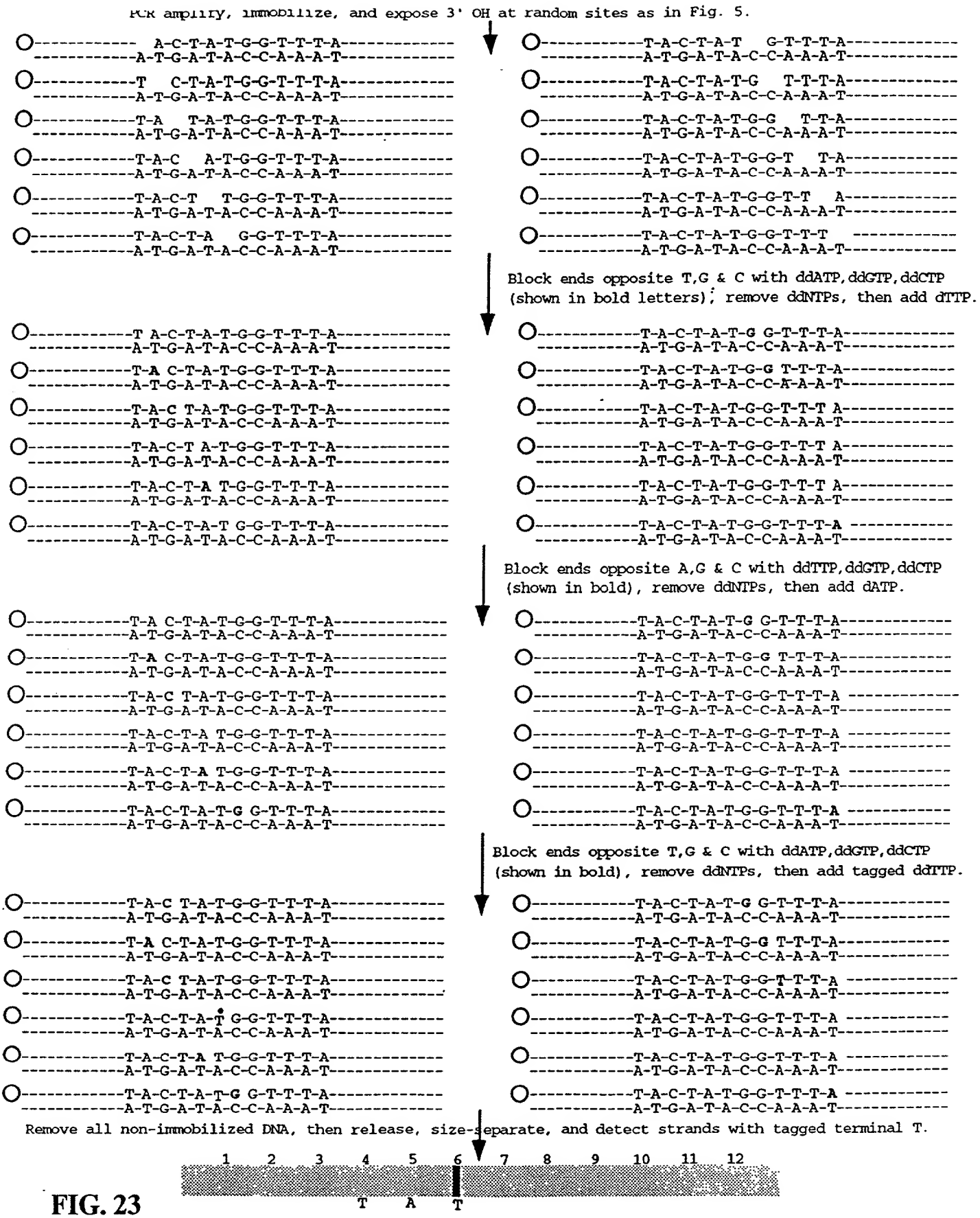

PCR amplify, immobilize, and expose 3' OH at random sites as in Fig. 5.
Block ends opposite T,G & C with ddATP,ddGTP,ddCTP (shown in bold letters); remove ddNTPs, then add dTTP.
Block ends opposite A,G & C with ddTTP,ddGTP,ddCTP (shown in bold), remove ddNTPs, then add dATP.
Block ends opposite T,G & C with ddATP,ddGTP,ddCTP (shown in bold), remove ddNTPs, then add tagged ddTTP.
Remove all non-immobilized DNA, then release, size-separate, and detect strands with tagged terminal T.
1 2 3 4 5 6 7 8 9 10 11 12
T A T

FIG. 23

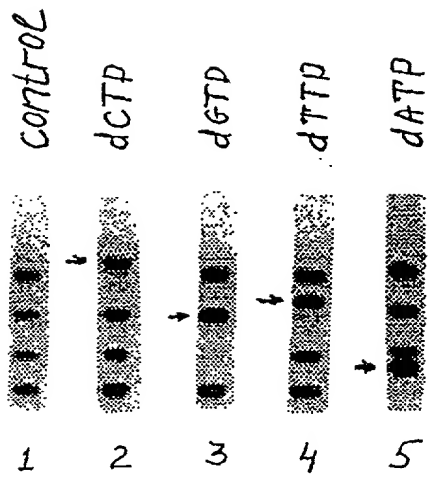


FIG. 24

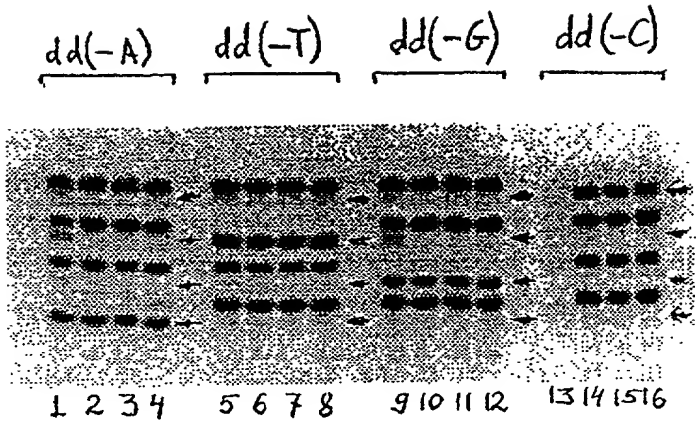


FIG. 25

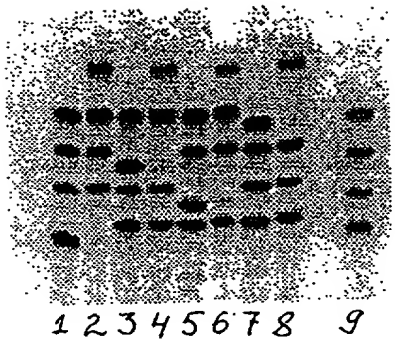


FIG. 26

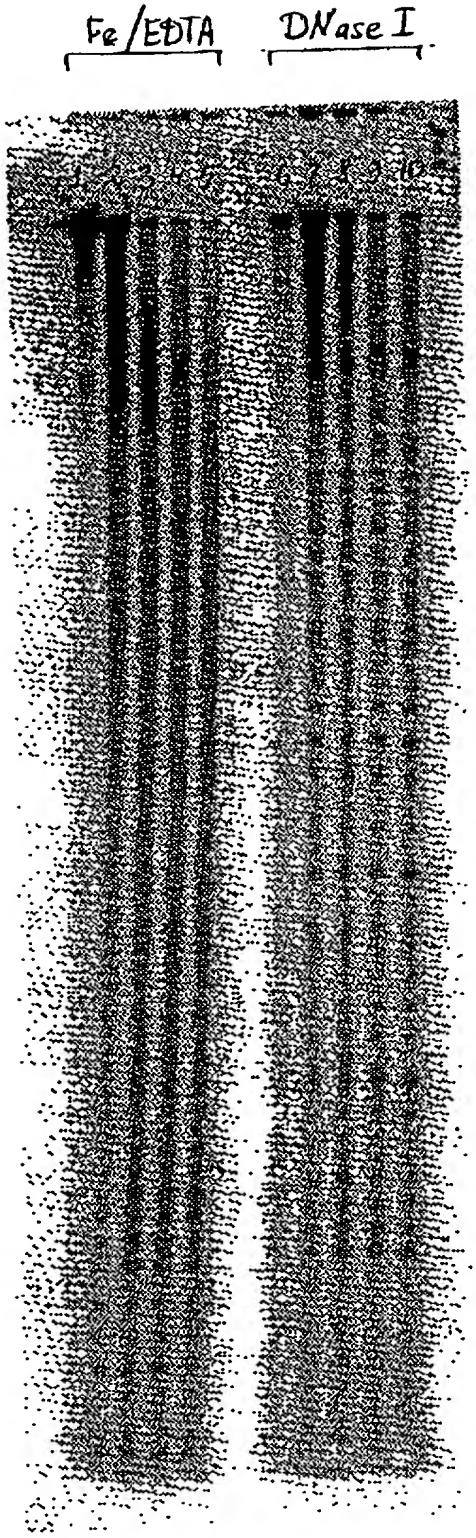


FIG. 27

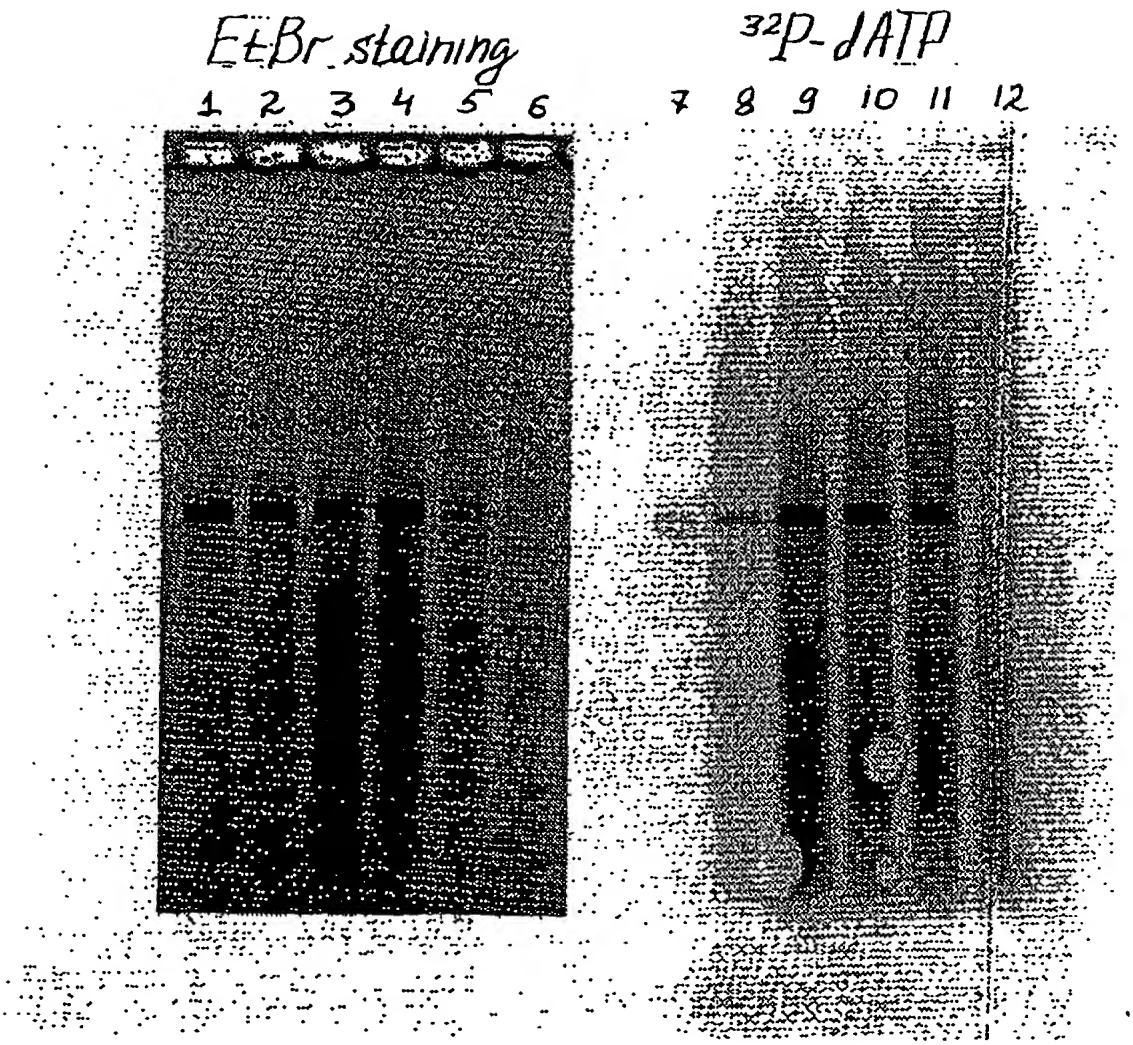


FIG. 28A

FIG. 28B

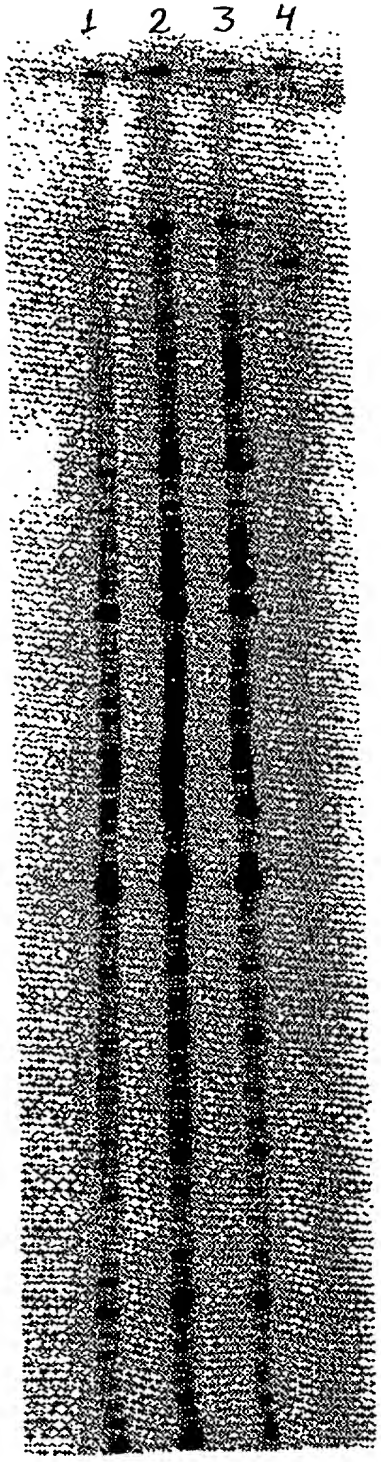
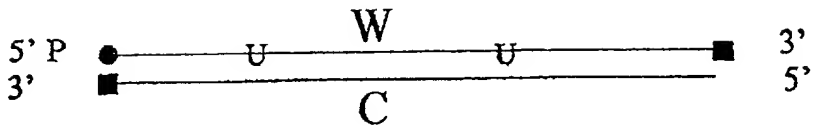


FIG. 29

FIG. 30A



● - 5' -phosphate

■ - 3' dideoxynucleotide or NH_3 group

5' ———	X	3' OH	4 C-X oligos
5' ———	XY	3' OH	16 C-XY oligos
5' ———	XYZ	3' OH	64 C-XYZ oligos

X, Y and Z are A, T, G or C

FIG. 30B

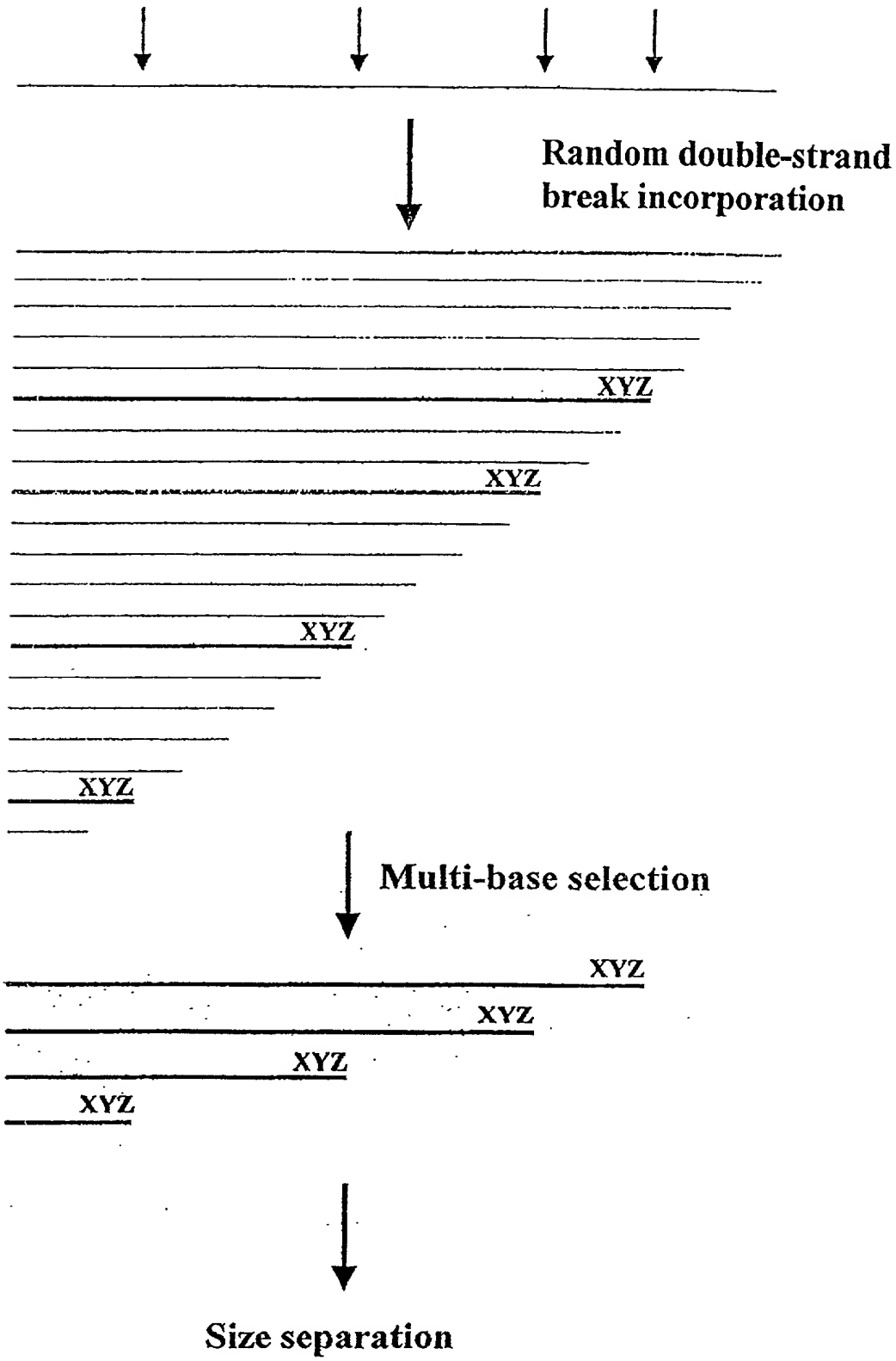

Map of the XYZ sites
Random double-strand break incorporation
XYZ
XYZ
XYZ
XYZ
Multi-base selection
XYZ
XYZ
XYZ
XYZ
Size separation

FIG. 31

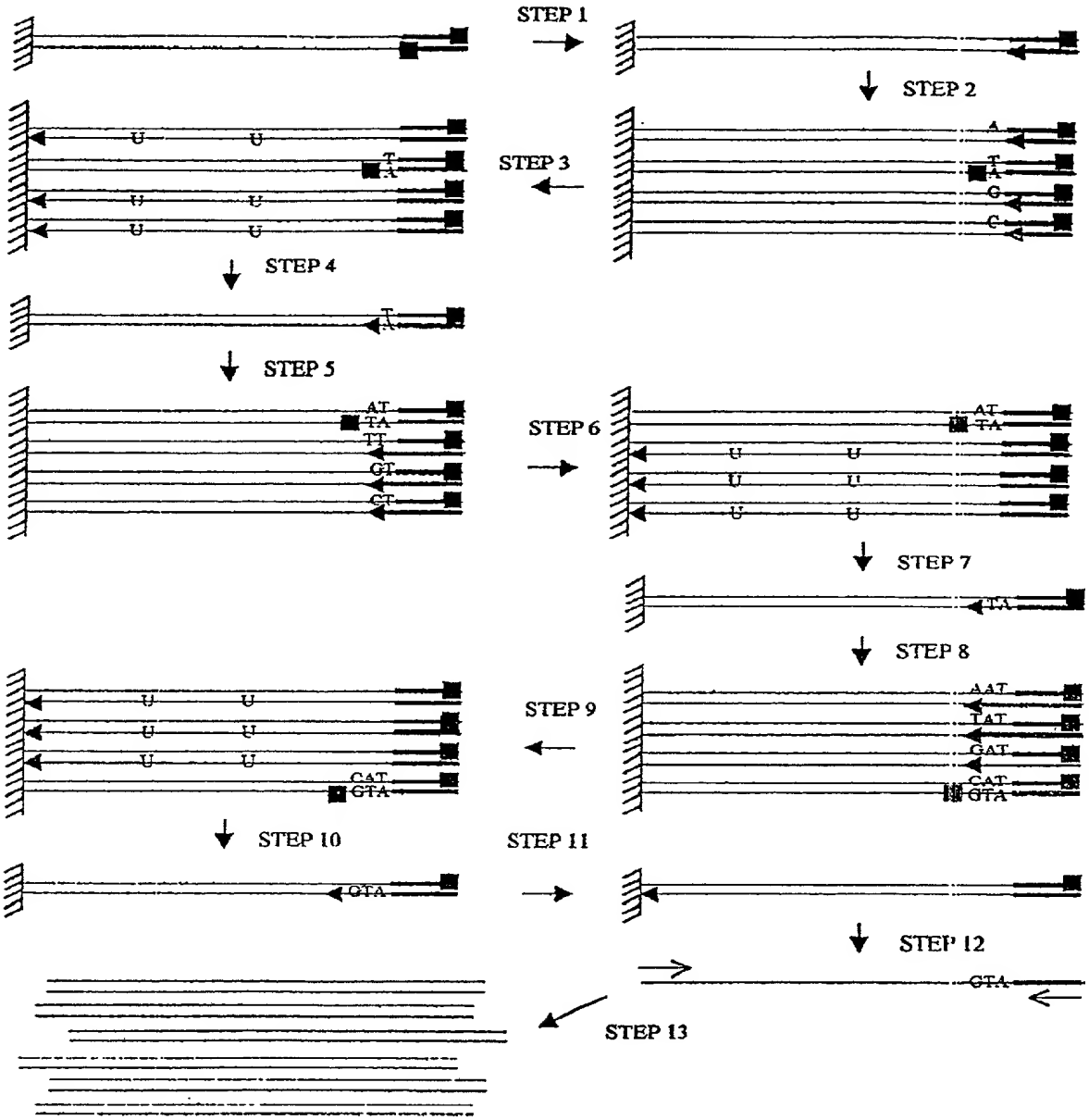

STEP 1
STEP 2
STEP 3
STEP 4
STEP 5
STEP 6
STEP 7
STEP 8
STEP 9
STEP 10
STEP 11
STEP 12
STEP 13

FIG. 32

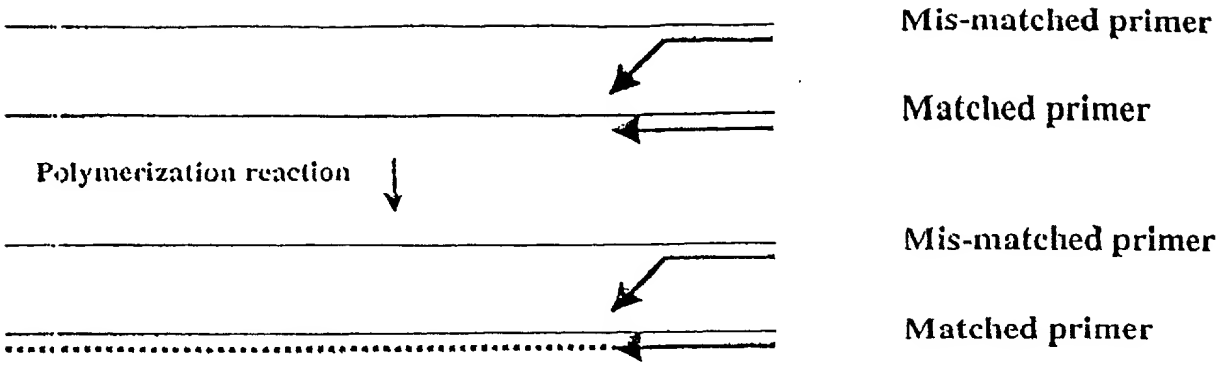


FIG. 33A

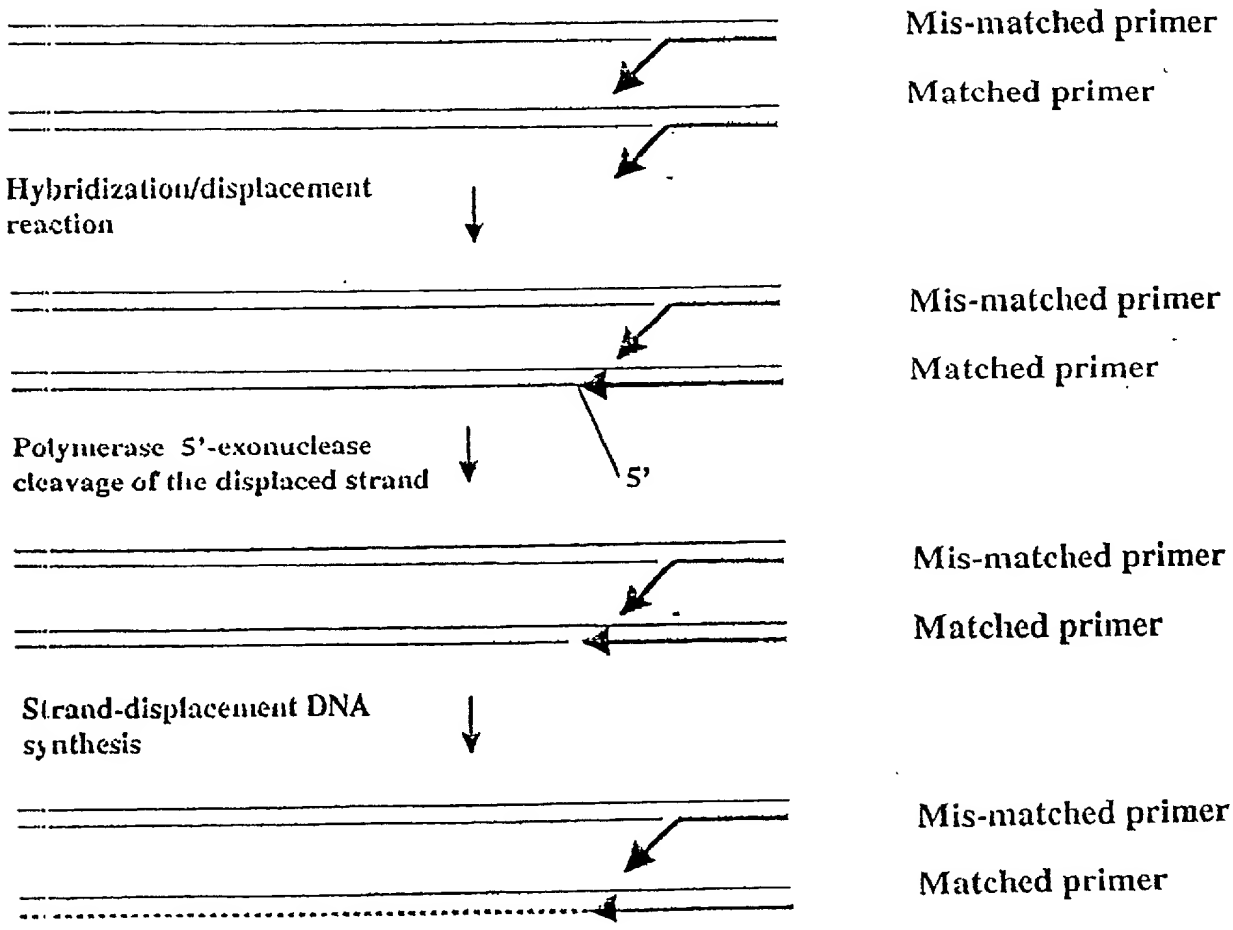


FIG. 33B